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(54) Title: PROCESS FOR THE DEPYROGENATION OF INJECTABLE PHARMACEUTICAL SOLUTIONS

(57) Abstract

The present invention relates to a process for the preparation of injectable solutions of pharmaceutical products and/or of diagnostic agents, characterized by an extremely high degree of purity as regards the low content of bacterial endotoxins. The process of the invention provides for the prefiltering of the solutions by means of a microfiltering unit, a subsequent ultrafiltration and the recycling of the retentate from the ultrafiltration to the solution which emerges from the prefilter.

PROCESS FOR THE DEPYROGENATION OF INJECTABLE PHARMA-
CEUTICAL SOLUTIONS

The present invention relates to the preparation of
injectable solutions of pharmaceutical products and/or
of diagnostic agents, characterized by an extremely high
degree of purity as regards the low content of bacterial
5   endotoxins.

It is known that injectable formulations of
pharmaceutical products or of diagnostic agents must
satisfy stringent criteria of sterility and of
apyrogenicity, in order to be able to be administered to
10   patients with acceptable margins of safety. Accordingly
it is of fundamental importance to eliminate as
completely as possible every pathogen and also the
bacterial endotoxins, from the final formulation of the
active principle before making-up, in order to avoid
15   undesired and frequently hazardous reactions of the body
to said toxic agents.

This requirement is all the more perceived in the
preparation of diagnostic contrastographic formulations,
where there is frequently the need of administering to
20   the patient large volumes of highly concentrated
solutions of said formulations.

Among the various diagnostic imaging techniques (X-
ray, NMR, echography), it is worth mentioning, by way of
example, the X-ray technique in which the opacifying
25   contrastographic agent is preferably represented by a
non-ionic iodine-containing aromatic compound.

To provide a sufficient contrast, the injectable
solutions of these compounds are usually very

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concentrated, reaching approximately the value of 80% w/v, and, for example by the intravenous route, are administered in volumes which may reach 250 mL and above per single dose.

5   Another administration route adopted for these compounds is, for example, the intrathecal route for tests which concern the neural tissue and in this connection it is well known that this type of tissue proves to be very much more sensitive than the others to

10   the toxic agents (up to 1000 times more sensitive).

It is accordingly clear that, especially in injectable solutions of these compounds, the presence of bacterial endotoxins must be as low as possible.

Among those techniques for the depyrogenation of

15   liquids which are in common use industrially, the use of microporous filters and of ultrafiltration membranes has by now become widespread.

Such membranes are, at all events, widely used, with particularly satisfactory results, for the

20   treatment of water or of dilute solutions.

Unfortunately, the situation proves to be very different when it is desired to subject to ultrafiltration solutions of contrast media, such as in particular non-ionic iodine-containing compounds, which

25   are very concentrated and viscous.

The problem is that by reason of the characteristics of these solutions, there is a need for filtering surfaces which are very large, in order to remain within the range of practicable production times

30   which are industrially acceptable. By contrast, smaller surfaces would excessively extend the filtration times.

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Consequently, the plant required is bulky and characterized by significant dead volumes in which non-negligible quantities of unfiltered solution stagnate, which solution, at the end of each working cycle, has to be discarded or recovered and treated separately in a suitable smaller plant. All this has significant unfavourable effects on the total production costs and, potentially, also on the final quality of the product.

An obvious attempt at resolving this difficulty consisted in the use of spiral-wound membranes for tangential ultrafiltration (having a suitable porosity capable of blocking the passage of the bacterial endotoxins: for example, an average cut-off of 10,000 dalton) which are accomodated in cartridges of relatively restricted dimensions and capable of working under pressure, a possibility which for technical reasons is not applicable to other filtration systems, such as for example those formed by so-called string filters, used industrially in this field. In this way, it should have been possible to obtain substantial improvements in terms both of space and of process speed.

It was, however, unexpectedly found that the membranes which would theoretically have been best, especially with regard to the characteristics of strength and of service life, such as for example those which are polysulphone-based, not only became obstructed within more or less brief periods of time depending upon the characteristics of the pharmaceutical preparation to be filtered, but in addition showed the property of impairing the actual composition of the formulation of

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the contrast medium. In fact, the membrane partially rejected the saline excipient (especially when said excipient is EDTA.Ca2Na), preferentially transmitting the non-ionic iodine-containing agent. This phenomenon,

5   which was entirely unexpected and, according to the actual supplier of the membrane, unforeseeable for pharmaceutical solutions of this type, made it impossible to apply this technique for the desired purpose.

10   Nor could the performance of the membrane be restored by the frequent use of detergents, for example after each working cycle, as the aforementioned phenomenon was already manifested after the passage of a few hundred litres of solution. Nor did it prove

15   possible to resolve the problem in an acceptable manner by changing the type of filtering material. Finally, complex experimentation led to the identification of a class of membranes, in essence those which are cellulose-based, which enabled the desired pyrogen

20   retention to be obtained without impairing to a substantial extent the composition of the formulation. However, it was not possible to prevent said membrane from becoming obstructed in short lengths of time.

The insertion of prefilters upstream of the

25   ultrafiltration unit proved to be equally disappointing as, by reason of the particular characteristics of high concentration and viscosity of the solutions in question, in addition to the very fine microimpurities contained in these, said prefilters lost their

30   effectiveness by becoming obstructed prematurely under the pressure conditions necessary for obtaining

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acceptable prefiltration times. It was possible to
obviate this defect only by arranging a series of
prefilters connected in parallel, where said filters
were connected to one another by a complex bypass system

5   the function of which was to switch the solution to be
depyrogenated from one prefilter to the next, without
having on each occasion to interrupt the process in
order to replace the prefiltering cartridge which had
become obstructed. All this involved the construction of

10   a plant which was complex and bulky and the management
of which was sophisticated and costly. Furthermore, the
final yield of the process proved in every case to be
suboptimal by reason of the dead spaces due to the
prefiltration system. To demonstrate the situation in a

15   clearer fashion, a diagrammatic description of the plant
is shown in Fig. 1.

As indicated therein, the solution to be
depyrogenated is driven from the feed-tank A through the
prefilter to the collection tank D under pressure

20   conditions such as to ensure total flow rates of
1200/1500 and even more L/h, irrespective of the
prefilter used (a substantially lower flow rate would
not be acceptable in the general economics of the
process, involving excessively long total filtration

25   time).

These conditions of high pressure, especially with
highly viscous solutions, cause the rapid obstructing of
the prefilter, which would need to be replaced on a
plurality of occasions for each working cycle. As a

30   consequence, there would be an extension of the overall
time, loss of product and the possibility of

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contamination.

The adoption of a series of prefilters (C1, C2, C3) connected in parallel and controlled by a bypass (B) which diverts from one filter to the next the solution arriving from A permits the completion of the working cycle in the times and under the working conditions which are desired, without having to interrupt the process.

Naturally, the disadvantageous aspects of this process are connected with the complexity of the prefiltering system, with the space necessary to accommodate the same and the high maintenance costs, bearing in mind that exhausted filters have to be removed and replaced by new ones. These problems assume ever greater importance the more substantial is the batch to be purified, as the number of cartridges to be used for each cycle is dependent both upon the working conditions (for example, the pressure applied and the temperature), and also upon the total volume of the solution to be treated.

On the other hand, the possible alternatives consisted in the adoption either of a very much larger and bulkier prefilter or of substantially lower pressure values, both parameters not being applicable to industrial processes involving batches of at least 800-1500 L of formulation.

The prefiltered solution is collected in a second tank D, from which it is subsequently taken off and pumped to the filtering unit E in accordance with the procedures which are normally employed in ultrafiltration. The depyrogenated filtrate ends up in a

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suitable collector where, before being made up, it will
be rehomogenized under agitation, while the residue,
progressively enriched in pyrogens, is fed back to the
tank D, being diluted, and then recycled to the filter E

5   until such time as the entire process is completed.

It has now unexpectedly been found that it is
possible to solve in an optimal fashion the above
discussed problems by virtue of a process which can be
carried out by means of a very simple filtration plant,

10   of modest dimensions, which plant can be managed in an
economic fashion and is capable of giving a high total
yield both in terms of quality and in terms of quantity.

The process of the invention comprises the
following steps:

15   -   prefiltration of said solutions by means of a
microfiltering unit,
-     passing of the solution deriving from the preceding
step to the ultrafiltration unit, in which said
ultrafiltration unit is equipped with tangential-

20   ultrafiltration membranes having a porosity such as
to deny passage to bacterial endotoxins and in which
the retentate from said unit is directly recycled to
the solution which emerges from the prefilter, while
the permeate, complying with the limits set by the

25   pharmacopoeia for the pyrogen content, is collected
in a collector, where it is homogenized under
agitation before being made up.

The process of the invention may conveniently be
carried out using a plant, diagrammatically illustrated

30   in Fig. 2, which is extremely compact as it uses only a
single prefiltration unit C, and unexpectedly does not

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require a tank for collecting the prefiltrate. The prefilter may take various forms, for example one or more filtering cartridges of decreasing porosity connected in series with one another. By way of example, a preferred configuration may comprise two filtering cartridges, of 1 μ and 0.22 μ respectively. Equally preferred is for example a prefilter constituted by a single composite filtering cartridge, that is to say one having decreasing porosity, with an average porosity of 0.5 μ. In substance, the solution to be depyrogenated is driven through the prefilter C by virtue of a modest pressure applied from the exterior to the tank A (acceptable values range from 1 to 3 atm, preferably from 1.5 to 2.5 atm).

As it emerges from the prefilter, the solution is pumped directly to the ultrafiltration unit: the depyrogenated permeate is collected in a suitable collector provided with agitation and the retentate is recycled directly upstream of the pump which manages the ultrafiltration, to be admixed with the solution passing out from the prefilter. The ultrafiltering unit may be variously constituted: one of the preferred configurations may, for example, comprise a plurality of filtering modules (housings), in their turn comprising one or more filtering cartridges, said housings being capable of operating simultaneously or individually, depending upon the quantity of solution to be filtered. The membranes which are preferably used are those which are capable of preventing a substantial impairment of the composition of the formulation during the process (for example, permitting the preferential passage of one

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or more constituents as compared with the others) and having a porosity such as to prevent the passage of bacterial endotoxins.

From this point of view, cellulose-based membranes have proved to be particularly preferred, especially those of regenerated cellulose, with an average cut-off of 10,000 dalton. An absolutely non-limiting example of these is represented by spiral-wound cartridges of regenerated cellulose S10Y10$^{(R)}$ and/or S40Y10$^{(R)}$ (Amicon).

The transmembrane pressure applied to the ultrafiltration unit varies, depending upon the type of filtering cartridge employed and upon the characteristics of the solution to be filtered (by way of example, a very important factor is the concentration and thus the viscosity of said solution).

As a general rule, entirely acceptable results are obtained by applying transmembrane pressures which are variable between 1 and 5 atm. Naturally, when it is necessary to depyrogenate less viscous solutions, it will be possible to obtain high flow rates (and therefore shorter times) when applying a lower transmembrane pressure.

The flow rates which are obtainable may normally vary between 4 L/h per m^2 of filtering surface and 70 L/h·m^2, preferably between 6 and 55 L/h·m^2.

The operating temperature is another important parameter, and is dependent upon the thermal stability of the diagnostic formulation to be filtered and upon the limits imposed by the filtering material. In the case of cellulose-based membranes, particularly with

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those of regenerated cellulose, it is possible to operate at from 20° to 55°C, for example. Particularly preferred, in the case of highly concentrated and viscous solutions, is a temperature of approximately 50-
5   54°C, preferably 52°C ± 2°C, while in the case of less viscous solutions it is preferred to operate at temperatures of approximately 30°C.

An absolutely non-limiting example is represented by the filtration of batches of 100-200 L of Iomeron[R]
10   150 (an injectable speciality containing the non-ionic iodine-containing contrast agent iomeprol in a concentration corresponding to a content of 150 mg/I per mL) in an AMICON SP150 + 52062 ultrafiltration unit equipped with S4010[R] ultrafiltering cartridges
15   (Amicon) for a total filtering surface of approximately 11 m^2. Working at a temperature of 45°C ± 5°C and applying a transmembrane pressure which is variable from batch to batch from 1 to ~4 atm, flow rates were obtained which were within the range between
20   approximately 12 and 45 L/h per m^2 with filtration times (for batches of 200 L) within the range between 0.4 and 1.5 h.

Another example of the invention may be represented by the depyrogenation of an industrial batch of 1,000 L
25   of Iopamiro[R] 300 (an injectable speciality containing the non-ionic iodine-containing contrast agent iopamidol in a concentration corresponding to a content of 300 mg/I per mL) carried out in an Amicon SPM480 unit equipped with cartridges of regenerated cellulose for a
30   total filtering surface of approximately 45 m^2, average cut-off of 10,000 dalton. Working at a temperature of

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52°C ± 2°C and applying a transmembrane pressure of 3.5 atm, a flow rate of ~11 L/h per m² is obtained for a total filtration time of approximately 2 h.

The plant constructed proved to be surprisingly efficient, especially as, with the elimination of the tank D (typical of an ultrafiltration process: as a common practice, where there are no prefiltration problems, the residue is directly recycled, becoming diluted, to the feed tank, something which is obviously not possible in the present situation), the retentate c, becoming admixed directly with the flow arriving from the prefilter, causes an almost immediate increase in the concentration of toxins in the solution to be filtered. This fact should obviously impair the efficiency of the filtering unit.

However, the efficiency of the process proves to be optimal and the actual life of the cellulose filter proves to be surprisingly long, whereas its greater structural delicacy had initially greatly discouraged the continuous use thereof for the filtration of highly viscous solutions, such as for example X-ray diagnostic solutions, and especially at temperatures which are very close to the operating limits prescribed for said filter.

The fact that the process is a single-stage process eliminates the time wasted in collecting the prefiltrate in the tank D of Fig. 1. It further proves to be possible to operate at moderate operating pressures, with obvious benefits regarding the safety of the plant.

There is thus created a single-stage process (on the contrary the one illustrated in Fig. 1 is in essence

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a two-stage process) which is economic, is in full compliance with the most stringent regulatory standards, is highly efficient and is also entirely in line with the requirement to show care for the environment because

5   it eliminates the need to use detergents to clean and restore the filtering surfaces (a simple step of sanitizing said surfaces, for example with soda, is sufficient). This gives a drastic improvement from the point of view of the impact on the environment.

10   Furthermore, this also removes the need to eliminate every residual trace of detergent and the need then to analytically certify its absence. All this brings clear benefits in terms of costs and quality of the finished product as well.

15   If required, it is also possible to insert at the outlet, preferably downstream of the collector/mixer of the filtrate (not indicated in Fig. 2), a further sterilizing filter of 0.2 µ or even less (F, indicated in the outlined zone of Fig. 2), thus obtaining a final

20   solution which is homogeneous and entirely sterile, ready to be made up.

          The flow rate remains substantially constant throughout the entire duration of the process. In this way, it remains possible to treat preparations up to

25   800-1,500 L in modest times (1.5-5 h) with yields of up to 97-98%, obtaining a product which is in full compliance with the most stringent pharmacopoeia limits.

          The plant of Fig. 2 has proved to be particularly useful for the depyrogenation of diagnostic injectable

30   pharmaceutical formulations. Radio-opaque diagnostic formulations comprising as contrast agent a non-ionic

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iodine-containing compound or a mixture of iodine-
containing compounds, either or monomeric or of dimeric
type, have proved to be particularly preferred.

Absolutely non-limiting examples of iodine-
containing radio-opaque contrast agents, injectable
formulations of which can be depyrogenated by means of
the process and the plant of the invention, may be
selected from among the following monomeric and dimeric
compounds and mixtures thereof: iopamidol, iomeprol,
iohexol, ioversol, iopentol, iopromide, ioxilan,
iotriside, iobitridol, iodixanol, iofratol, iotrolan,
iodecimol, iopirol, iopiperidol.

Equal preference has proved to be ascribable to the
injectable formulations of contrast agents for NMR
imaging, usually comprising, as contrastographic
ingredients, chelates of paramagnetic metal ions (such
as for example Gd^{3+}, Mn^{2+}, Fe^{3+}, Eu^{3+}, Dy^{3+} etc.) with
chelating agents of various types (such as for example
linear or cyclic polycarboxylic polyamino acids, and
derivatives or salts thereof, polyaminophosphonic or
polyaminophosphinic acids and derivatives thereof etc.).

Absolutely non-limiting examples of contrast agents
of this type may be selected from among the following:
chelate of Gd^{3+} with diethylenetriamine pentaacetic acid
(Gd-DTPA), chelate of Gd^{3+} with 1,4,7,10-
tetraazacyclododecan-1,4,7,10-tetraacetic acid (Gd-
DOTA), chelate of Gd^{3+} with [10-(2-hydroxypropyl)-
1,4,7,10-tetraazacyclodecan-1,4,7-triacetic acid (Gd-
HPD03A), chelate of Gd^{3+} with 4-carboxy-5,8,11-
tris(carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatride-
can-13-oic acid (Gd-BOPTA), chelate of Gd^{3+} with N-[2-

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[bis(carboxymethyl)amino]-3-(4-ethoxyphenyl)propyl]-N-
(2-[bis(carboxymethyl)amino]ethylglycine   (Gd-EOB-DTPA),
chelate of Gd^{3+} with N,N-bis[2-[(carboxymethyl)[(me-
thylcarbamoyl)methyl]amino]ethyl]-glycine (Gd-DTPA-BMA),
chelate   of Gd^{3+} with   (α,α',α'',α''')-tetramethyl-
1,4,7,10-tetraazocyclododecan-1,4,7,10-tetraacetic   acid
(Gd-DOTMA), chelate of Mn^{2+} with N,N'-bis(pyrodoxal-5-
phosphate)ethylene-diamine-N,N'-diacetic acid (Mn-DPDP)
and salts or derivatives thereof, such as for example
those of amide or ester type.

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CLAIMS

1. Process for the depyrogenation of injectable
pharmaceutical solutions, substantially comprising the
following steps:
- prefiltration of said solutions by means of a
  microfiltering unit,
- passing of the solution deriving from the preceding
  step to the ultrafiltration unit, in which said
  ultrafiltration unit is equipped with tangential-
  ultrafiltration membranes having a porosity such as
  to deny passage to bacterial endotoxins and in which
  the retentate from said unit is directly recycled to
  the solution which emerges from the prefilter, while
  the permeate, complying with the limits set by the
  pharmacopoeia for the pyrogen content, is collected
  in a collector, where it is homogenized under
  agitation before being made up.

2. Process according to Claim 1, in which the
injectable pharmaceutical solution is passed to the
prefilter under a pressure which is variable between 1
and 3 atm.

3. Process according to Claim 1, in which the prefilter
comprises 1 or more filtering cartridges of decreasing
porosity connected in series with one another.

4. Process according to Claim 3, in which the prefilter
comprises two filtering cartridges which are connected
in series, of 1 µ to 0.22 µ respectively.

5. Process according to Claim 3, in which the prefilter
comprises a single filtering cartridge of decreasing
porosity, having an average porosity of 0.5 µ.

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6. Process according to Claim 1, in which the ultrafiltration unit comprises one or more filtration modules, which are equipped with tangential-ultrafiltration membranes having an average cut-off of 10,000 dalton, which are capable of operating simultaneously or individually.

7. Process according to Claim 6, in which said tangential-ultrafiltration membranes are substantially cellulose-based.

8. Process according to Claim 7, in which said membranes are of regenerated cellulose.

9. Process according to Claim 1, in which the transmembrane pressure applied to the ultrafiltration unit permits an outflow rate of the permeate which is variable between 4 and 70 L/h per m^2.

10. Process according to Claim 9, in which said transmembrane pressure is variable between 1 and 5 atm.

11. Process according to Claim 1, in which the operating temperature is variable between 20° and 55°C throughout the entire process.

12. Process according to Claim 1, in which said injectable pharmaceutical solution is a formulation for diagnostic use.

13. Process according to Claim 12, in which said diagnostic pharmaceutical formulation comprises, as active principle, at least one non-ionic iodine-containing radio-opaque agent.

14. Process according to Claim 13, in which said radio-opaque agent is iopamidol.

15. Process according to Claim 13, in which said radio-opaque agent is iomeprol.

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16. Process according to Claim 13, in which said formulation comprises a mixture of monomeric and dimeric non-ionic iodine-containing radio-opaque agents.

17. Process according to Claim 13, in which said diagnostic pharmaceutical formulation comprises, as active principle, at least one contrastographic agent for NMR imaging.

18. Process according to Claim 17, in which said contrastographic agent is a chelate of the ion of a paramagnetic metal.

19. Process according to Claim 18, in which said paramagnetic chelate is selected from among: Gd-BOPTA and Gd-HPDO3A and salts thereof.

20. Plant for depyrogenating, by means of tangential ultrafiltration, injectable pharmaceutical solutions according to Claim 1, said plant substantially corresponding to Fig. 2 and to the pertinent description.

21. Plant according to Claim 20 for the depyrogenation of diagnostic injectable pharmaceutical solutions.

22. Plant according to Claim 20 for the depyrogenation of radio-opaque injectable diagnostic solutions.
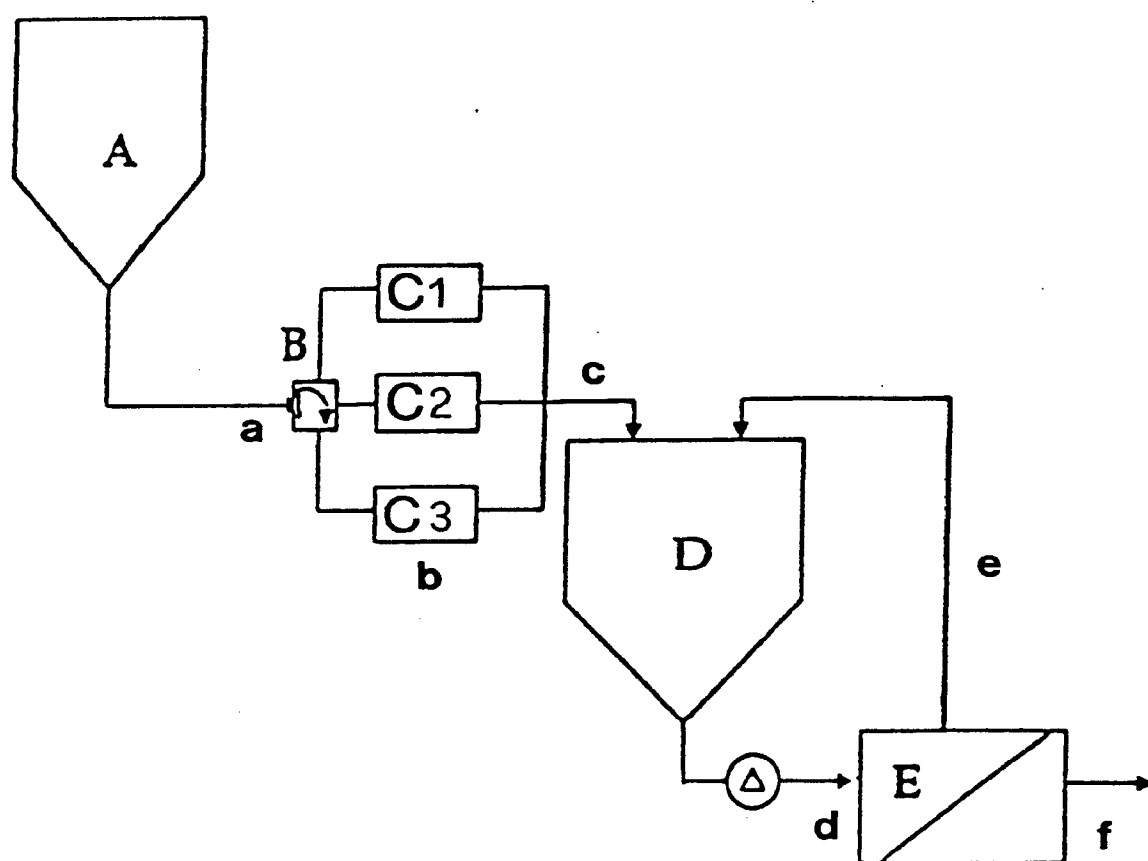
23. Plant according to Claim 20 for the depyrogenation of injectable diagnostic solutions for NMR imaging.

24. Depyrogenated radio-opaque injectable diagnostic solutions obtained according to the process of claim 1.

25. Depyrogenated injectable diagnostic solutions for NMR imaging obtained according to the process of claim 1.
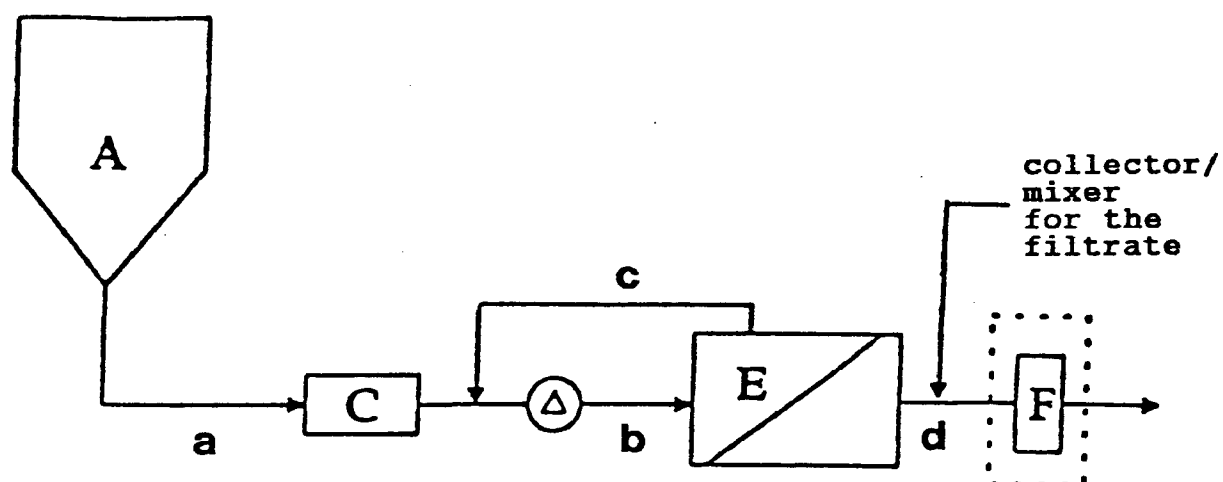
FIGURE 1

FIGURE 2

collector/
mixer
for the
filtrate

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61L2/00   B01D61/14   A61K9/00   A61K9/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61L   B01D   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	EP,A,0 312 104 (TANABE SEIYAKU CO.,LTD) 19 April 1989 see page 8, line 14-17; claims 2-4; figures 8,9 ---	1-6,9-25
Y	DATABASE WPI Week 9210 Derwent Publications Ltd., London, GB; AN 92-077012 XP002012908 & JP,A,04 022 491 (NOK CORP) , 27 January 1992 see abstract --- -/--	1-6,9-25

X	Further documents are listed in the continuation of box C.		X	Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
9 September 1996	2 0. 09. 96

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

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		CA-A-	2084873	30-12-91
		EP-A-	0536297	14-04-93
		JP-T-	6507375	25-08-94
		US-A-	5221483	22-06-93
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		CA-A-	2080939	29-01-94
		DE-A-	4325335	03-02-94
		FR-A-	2694282	04-02-94
		JP-A-	6154752	03-06-94
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		JP-A-	59149901	28-08-84
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